Enzymic acylation of glutamine by phenylacetic acid

The excretion of phenylacetylglutamine (PAG), a constituent of normal human urine[1], is augmented by oral administration of phenylacetate[2]. Considerable quantities of PAG have also been found in the urine of patients with phenylpyruvic oligophrenia[1,3,4]. The available evidence suggests that the phenylacetyl moiety of PAG excreted in this disease and by normal individuals is derived from phenylalanine. In contrast, the benzoyl moiety of hippuric acid appears to arise mainly from dietary benzoate. Furthermore, although administered benzoate or phenylacetate is characteristically excreted by most mammals as the corresponding acyl glycine derivative, in man phenylacetate and benzoate follow different pathways, the former yielding PAG and the latter hippurate. The capacity to form PAG therefore appears to be restricted to human tissues and possibly also to those of the chimpanzee[5].

This communication describes an investigation of the enzymic mechanism involved in the formation of PAG by human tissues. When homogenates of human liver were incubated with ^{14}C-L-glutamine and phenylacetate, evidence was obtained for the formation of PAG. PAG synthesis was increased by addition of coenzyme A and ATP, suggesting that phenylacetyl-coenzyme A is an intermediate in the formation of PAG. Experiments were therefore carried out in which ^{14}C-L-glutamine was incubated with phenylacetyl-coenzyme A in the presence of several human tissues (Table I). After incubation, the reaction mixtures were brought to 70% with respect to ethanol and an aliquot of the ethanol-soluble fraction was applied to filter paper strips. After chromatography in *n*-butanol–water–acetic acid (4:1:1), 1 cm sections of the strips were counted with a thin mica-window tube. Under these conditions, PAG was readily separated from pyrrolidone carboxylic acid, α-ketoglutaric acid, glutamic acid, and glutamine. Formation of radioactive PAG was observed with human liver and kidney preparations, but could not be demonstrated with rat liver (Table I).

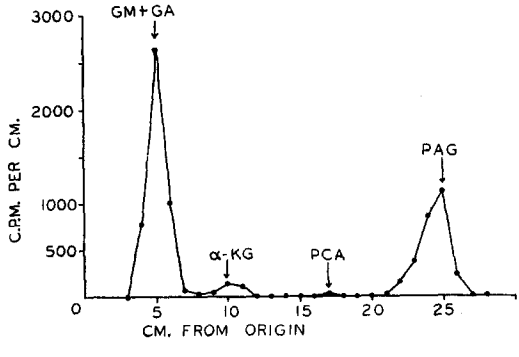
TABLE I

In vitro FORMATION OF ^{14}C-PHENYLACETYLGLUTAMINE BY HUMAN LIVER*

Source of homogenate	*% Conversion*
Human liver (biopsy)	9.8
Human liver (biopsy); boiled, 100°, 2 min	0
Human liver (biopsy); phenylacetyl-CoA omitted	0
Human liver (autopsy)	4.9
Human kidney (autopsy)	13.5
Rat liver	0

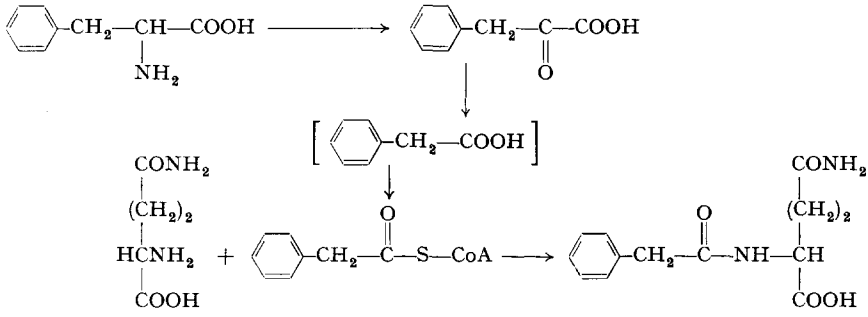
* The reaction mixture contained 0.6 ml of a 33% homogenate, 2.25 μmoles phenylacetyl-CoA, 1.26 μmoles randomly-labeled ^{14}C-L-glutamine and 150 μmoles sodium phosphate at pH 8.2 in a final volume of 1 ml; incubated for 1 h at 37.5°. Values are expressed as conversion of ^{14}C-glutamine to PAG.

Fig. 1. Radioactive analysis of a chromatogram of an ethanol-soluble fraction in *n*-butanol-acetic acid. Eight mg of a 40-fold purified human liver enzyme were incubated with 2.25 μmoles phenylacetyl-CoA, 1.26 μmoles ^{14}C-L-glutamine and 150 μmoles sodium phosphate at pH 8.2 for 1 h in a final volume of 1 ml and processed as described in the text. Chromatography in *tert.*-butanol-formic acid, in which glutamine and glutamic acid were resolved, indicated that approximately 30% of the ^{14}C-glutamine (GM) had been converted to glutamic acid (GA). A small amount of α-ketoglutaric acid (α-KG) was formed. The formation of greater amounts of pyrrolidone carboxylic acid (PCA) from glutamine, which occurs at elevated temperatures, was prevented by evaporating the ethanol-soluble fraction on filter paper with a stream of cold air.



Human liver and kidney were fractionated to yield preparations of the acylating activity that were 40- to 100-fold more active per milligram of protein than the original homogenates. A typical experiment carried out with the purified liver enzyme is described in Fig. 1. When chromatography was carried out with four other solvents, similar results were obtained, and within experimental error, the same amount of ^{14}C-PAG was recovered. In all five solvent systems, the R_F values of ^{14}C-PAG agreed with those of an authentic sample of PAG prepared by organic synthesis from L-glutamine. Elution of the ^{14}C-PAG from the paper strips, followed by acid hydrolysis, yielded glutamic acid as the only radioactive product.

The present results indicate that phenylacetyl-coenzyme A is an intermediate involved in PAG synthesis. Phenylacetyl-coenzyme A may arise in the course of the oxidative-decarboxylation of phenylpyruvate formed from phenylalanine by transamination, or by the activation of exogenous phenylacetate. These reactions may be represented as follows:



Studies on the specificity and other properties of the purified acylating enzyme system obtained from liver and kidney and of the activating system prepared from human liver mitochondria are in progress.

This investigation was supported in part by research grants from the U.S. Public Health Service and the National Science Foundation. The authors thank Dr. CHARLES G. CHILD for his generous assistance in providing biopsy samples and Dr. G. J. GHERARDI for his cooperation in providing the autopsy material.

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Received March 30th, 1957

Specific protection of the thiol groups of aldehyde dehydrogenases by pyridine-nucleotide coenzymes*

The potassium-activated yeast aldehyde dehydrogenase (BLACK[1]), the TPN-linked yeast aldehyde dehydrogenase (SEEGMILLER[2]), and the aldehyde dehydrogenase from liver (RACKER[3]), are inhibited by the following sulfhydryl reagents: a trivalent arsenical compound (3-amino-4-hydroxyphenylarsenoxide or *mapharside*), N-ethylmaleimide, *o*-iodosobenzoate, *p*-chloromercuribenzoate and iodoacetate. The yeast TPN-specific enzyme is comparatively the most sensitive to *p*-chloromercuribenzoate, *o*-iodosobenzoate, N-ethylmaleimide, and iodoacetate, whereas the yeast potassium-activated dehydrogenase is the most sensitive to *mapharside*. These findings allow the inclusion of yeast TPN-specific dehydrogenase in the group of SH enzymes and extend the already existing evidence of the essential role of thiols in the aldehyde dehydrogenases described by BLACK[1] and RACKER[3].

* DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.